be significantly different from their wild-type counterparts. This could be due to stimulation of the wild-type cells with unidentified endogenous TLR agonists, or to stimulation with trace LPS in culture media. In at least one study, microarray analysis of gene expression in resting wild-type and MyD88$^{-/-}$ bone marrow macrophages revealed significant differences that might be anticipated to affect phagocytosis and inflammatory responses to microbes independent of microbe-stimulated TLR activation (Shi et al., 2003). An unfortunate consequence then of relying solely on TLR/MyD88 knockout cells would be that one could make conclusions on the "acute TLR dependence" of a cellular function that is actually sensitive to the preexisting state of the cell. These differences may not be consistent between laboratories due to differing culture conditions.

The present study clearly demonstrates that TLR signaling does not necessarily modify the rate of phagosome maturation. However, it looked only at one type of macrophage, and it is still possible that TLR signaling modifies phagosome maturation rates after activation of the cells or in other types of phagocytes. For example, IFN-γ treatment of macrophages significantly modifies responses to TLR activation, and could well set up a case in which TLR signaling regulates the rate of phagosome maturation. Similarly, the present study examined only resting bone marrow-derived macrophages, and it is possible that other phagocytes such as dendritic cells, resident peritoneal macrophages, or elicited peritoneal macrophages might behave differently. It is also possible that preexposure to TLR agonists would have altered the rate of phagosome maturation, even though TLR activation during the minutes required for phagosome formation and maturation did not. TLR signaling modifies expression of many genes that are involved in membrane traffic and lysosomal function.

Microbial recognition by macrophages occurs through a variety of different receptors that can trigger phagocytosis through different mechanisms. For example, the actin cytoskeletal structures assembled to internalize complement-opsonized particles are very different from those assembled to internalize IgG-opsonized particles (Allen and Aderem, 1996). It is difficult to rule out the possibility that maturation of phagosomes formed during specific types of phagocytosis might be more highly regulated by TLR signaling than others. Yates and Russell examined internalization of IgG-, mannose-, and phosphatidylserine-coated particles as well as formalin-fixed *S. aureus*. This covers a relatively broad range of phagocytic receptors, but certainly not all. Also, there is growing appreciation that maturation of individual phagosome varies somewhat in an apparently random nature (Griffiths, 2004; Henry et al., 2004). For example, some phagosomes are coated with phosphatidylinositol (3,4,5)-triphosphate for just minutes, while others retain the lipid signaling molecule for at least an hour. Such heterogeneity may be inherent "noise" in a highly redundant system where there are many effective maturation paths. Alternately, there may be several different functional types of phagosomes. For example, while some phagosomes may be simply charged with obliterating internalized particles, others may be stocked with different sets of proteases to maximize diversity in peptide generation. Also, different phagosomal maturation paths may make greater energy demands on the cell, which must be balanced against other functions such as cytokine production and motility. It is possible that TLR signaling on phagosomes could affect the balance of formation of certain classes of phagosomes not apparent in the current Yates and Russell study.

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Macrophage Polarization Comes of Age

Functional polarization of macrophages into M1 or M2 cells is an operationally useful, simplified conceptual framework describing the plasticity of mononuclear phagocytes. Genetic approaches have begun to shed new light on mechanisms underlying macrophage polarization and on the actual in vivo significance of polarized M2 cells (Rauh et al., 2005 [this issue of *Immunity*]).

Heterogeneity and plasticity are hallmarks of cells belonging to the monocyte-macrophage lineage (Gordon, 2003; Mantovani et al., 2002; Mantovani et al., 2004). Lineage-defined populations of mononuclear phagocytes have not been identified, but short-lived, circulat-

ing precursor monocyte subsets characterized by differential expression of the FcγRIII receptor (CD16) and of chemokine receptors (CCR2, CX3CR1, and CCR8), as well as by different functional properties, have been described. Macrophage plasticity is demonstrated by acquisition of distinct morphological and functional properties directed by particular tissues (e.g., the lung alveolar macrophage) and immunological microenvironment.

In response to cytokines and microbial products, mononuclear phagocytes express specialized and polarized functional properties (Gordon, 2003; Mantovani et al., 2004). Mirroring the Th1/Th2 nomenclature, many refer to polarized macrophages as M1 and M2 cells. Classically activated M1 macrophages have long been known to be induced by IFNγ alone or in concert with microbial stimuli (e.g., LPS) or cytokines (e.g., TNF and GM-CSF). IL-4 and IL-13 were subsequently found to be more than simple inhibitors of macrophage activation and to induce an alternative M2 form of macrophage activation (Gordon, 2003). M2 is a generic name for various forms of activated macrophages, excluding classic M1 cells but including cells exposed to IL-4 or IL-13, immune complexes, IL-10, glucocorticoid, or secosteroid (vitamin D3) hormones (Mantovani et al., 2004).

In general, M1 cells have an IL-12^{high}, IL-23^{high}, IL-10^{low} phenotype; are efficient producers of effector molecules (reactive oxygen and nitrogen intermediates) and inflammatory cytokines (IL-1β, TNF, IL-6); participate as inducer and effector cells in polarized Th1 responses; and mediate resistance against intracellular parasites and tumors. In contrast, the various forms of M2 macrophages share an IL-12^{low}, IL-23^{low}, IL-10^{high} phenotype with variable capacity to produce inflammatory cytokines depending on the signal utilized. M2 cells generally have high levels of scavenger, mannose, and galactose-type receptors, and arginine metabolism is shifted to production of ornithine and polyamines via arginase. Differential regulation of components of the IL-1 system (Dinarello, 2005) occurs in polarized macrophages, with low IL-1β and low caspase I, high IL-1ra, and high decoy type II receptor in M2 cells. M1 and the various forms of M2 cells have distinct chemokine and chemokine receptor repertoires (Mantovani et al., 2004). In general, M2 cells participate in polarized Th2 reactions; promote killing and encapsulation of parasites (Noel et al., 2004); are present in established tumors and promote progression, tissue repair, and remodelling (Wynn, 2004); and have immunoregulatory functions (Mantovani et al., 2004). Immature myeloid suppressor cells have functional properties and a transcriptional profile related to M2 cells (S. Biswas, L. Gangi, S. Paul, T. Schioppa, A. Saccani, M. Sironi, B. Bottazzi, A. Doni, D. Bronte, F. Pasqualini, L. Vago, M. Nebuloni, A.M., and A.S., unpublished data).

Profiling techniques and genetic approaches have shed new light on the M1/M2 paradigm. Transcriptional profiling has offered a comprehensive picture of the genetic programs activated in polarized macrophages, led to the discovery of new polarization-associated genes (e.g., Fizz and YM-1), tested the validity of the paradigm in vivo in selected diseases (S. Biswas, L. Gangi, S. Paul, T. Schioppa, A. Saccani, M. Sironi, B. Bottazzi, A. Doni, D. Bronte, F. Pasqualini, L. Vago, M. Nebuloni, A.M., and A.S., unpublished data), and questioned the generality of some assumptions. For instance, unexpectedly, arginase is not expressed prominently in human IL-13-induced M2 cells (Scotton et al., 2005). M2 cells express high levels of the chitinase-like YM-1. Chitinases represent an antiparasite strategy conserved in evolution, and there is now evidence that acidic mammalian chitinase induced by IL-13 in macrophages is an important mediator of type II inflammation (Zhu et al., 2004).

Macrophages have long been held to play a key role in tissue repair. Indeed, there is now direct genetic evidence, taking advantage of M-CSF-deficient mice, that macrophages transmit regenerative signals to injured epithelium in the colon (Pull et al., 2005). Taking advantage of a conditional ablation system of macrophages based on transgenic expression of diphtheria toxin receptor, scar-associated macrophages were found to orchestrate scarring and myofibroblast proliferation, as M2 cells do in vitro, as well as matrix degradation during recovery (Duffield et al., 2005). Thus, genetic approaches validate long-held views on the role of macrophages in tissue repair on the one hand, and, on the other hand, emphasize the difficulties of mouse-to-human extrapolation and of modeling in vitro the complex plasticity of macrophages.

Two recent studies offer genetic tools and approaches to investigate the actual in vivo function of M2 cells. Mice with a selective deficiency of the IL-4 receptor α chain in myelomonocytic cells were generated. On the basis of this genetic approach, M2 cells are essential during Schistosoma infection for protection against organ injury because they downregulate egg-induced inflammation (Herbert et al., 2004). SHIP is a negative regulator of PI3K pathway and dampens LPS responsiveness in macrophages, playing a role in endotoxin tolerance (Rauh et al., 2005). Macrophages obtained from SHIP$^{-/-}$ mice show M2 skewing (Rauh et al., 2005). Intriguingly, the M2 orientation of SHIP$^{-/-}$ macrophages is not an intrinsic autonomous property of the lineage but requires an extrinsic signal provided by a TGFβ-rich microenvironment, as well as aging of the animals. There is some controversy concerning the role of PI3K in TLR signaling in different cell types (Fang et al., 2004). However, PI3K activation may be a universal requirement for M2 differentiation and activation, and SHIP is a negative regulator of skewing. The SHIP$^{-/-}$ mice spontaneously develop a lung pathology consisting of consolidation, fibrosis, and deposition of crystals composed of the M2-associated chitinase-like protein YM-1. Moreover, a mouse tumor grew faster in the SHIP$^{-/-}$ mice than in controls. These results suggest that SHIP is a negative regulator of M2 skewing in vivo, and they are generally consistent with a role for polarized M2 macrophages in tissue remodeling and tumor promotion.

Polarization of macrophage function should be viewed as an operationally useful, simplified, conceptual framework describing a continuum of diverse functional states (Mantovani et al., 2004). The genetic approaches now available provide tools to dissect the actual in vivo function of polarized macrophages and to explore the

limitations of the M1/M2 paradigm to define the complexity and plasticity of mononuclear phagocytes.

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